

Epigenetic Signatures of Combat: Methylation Risk Scores Predict Blast and Trauma Exposure and Warfighter Neurocognitive Performance

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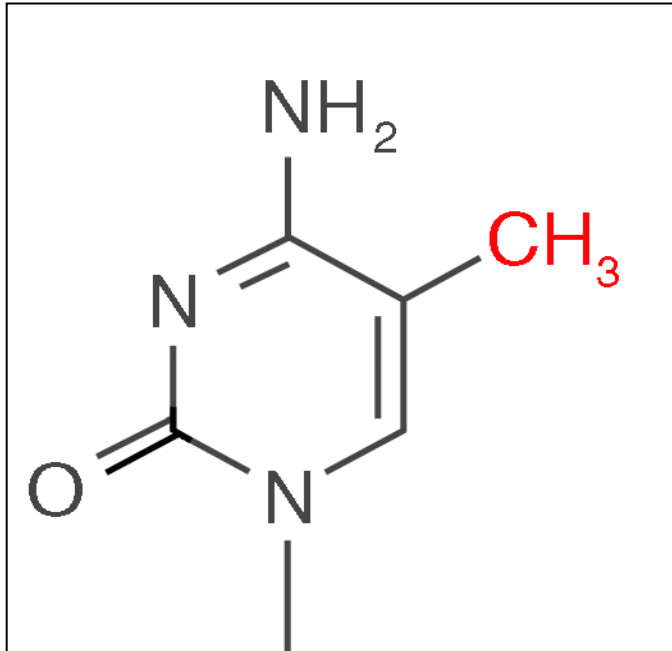
- Dr. Haghighi has no conflict of interest to report.
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Background & Overview

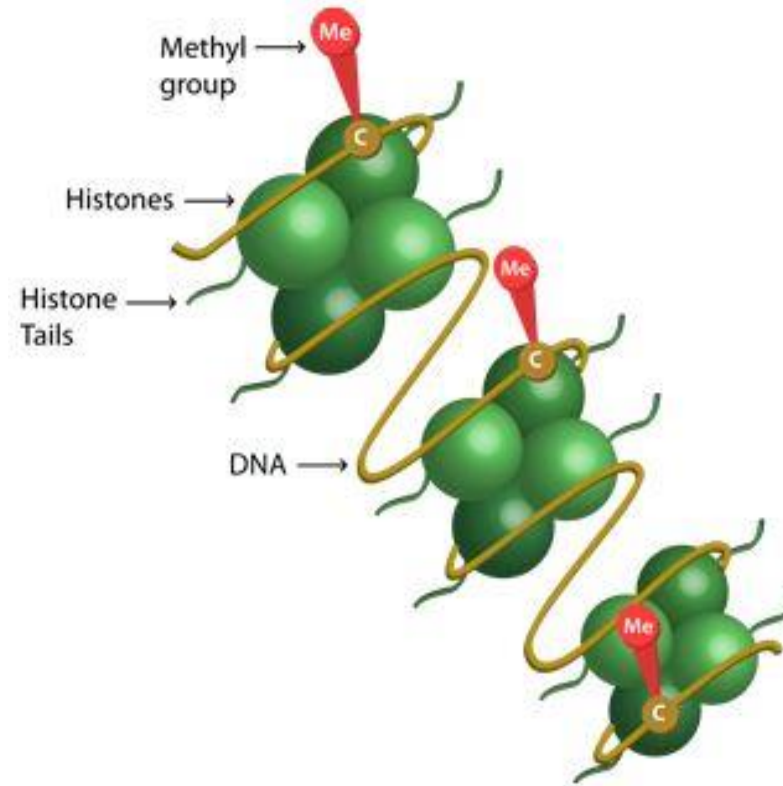
- Acutely: Exposures to low-level blast explosives during tactical training associate with transient concussion-like symptomology, and alterations in systemic transcriptional, inflammatory, gut permeability, and neurotrauma biomarkers.
- Chronically: Exposures to blast and trauma during military service can contribute to chronic clinical symptoms including:
 - Cognitive Impairment
 - Headaches
 - Sleep disturbances
 - PTSD
 - Depression,
 - Suicidality
- Repetitive exposures to blast and trauma cumulatively associate with evolving brain injury pathology detected through neuroimaging, neuropathological, and biomarker studies.
- **Question:** Do exposures from physical and/or psychological injuries during military service contribute to indelible epigenetic signatures of accelerated aging, transcriptional regulatory alterations, and neurocognitive performance in active-duty Warfighters?



DNA Methylation



Orstavik et al. 1995



- DNA methylation is a process in which methyl (Me, --CH_3) groups are added to molecules of DNA at cytosine residues, typically associated w/ repression of gene expression
- DNA methylation represents highly stable, long-lasting marks that encapsulate a lifetime of environmental exposures (stress, trauma, etc.)

Study Design & Methods

Participants:

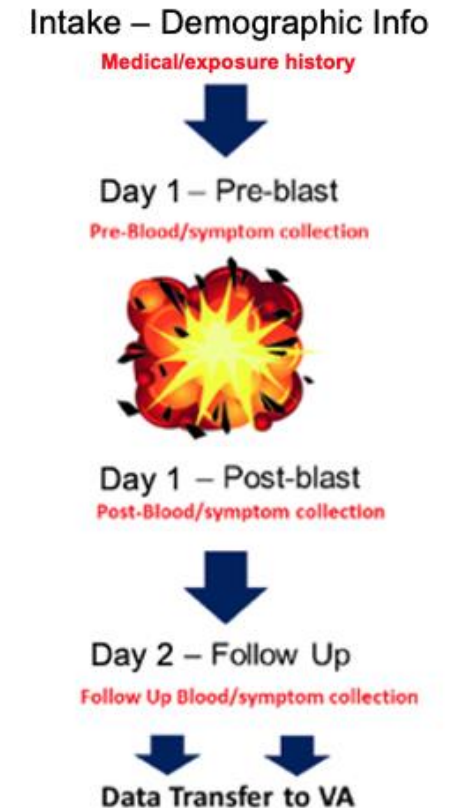
- Breachers (n=190): male service members engaged in heavy-wall breaching tactical operations and training across multiple cohorts (used in primary analyses).
- Hand grenade operators (n=30): male grenade instructors (used in validation analyses).

Data on medical/exposure and operational performance:

- Self-report data on SMS' combat exposure history obtained via Combat Exposure Scale (CES)*, and medical, TBI, and blast exposure history collected prior to training
- Neurocognitive performance during tactical training operations measured by Defense Automated Neurobehavioral Assessment (DANA) including subtasks: Simple Reaction Time, Procedural Reaction Time, Go/No-Go).
- Biospecimens: whole blood collected during operational trainings (used samples from pre-training timepoint).
- DNA Methylation: Profiled genome-scale DNA methylation assayed using Illumina Infinium HumanMethylation EPIC BeadChip covering genic and enhancer regions

Data and statistical analyses:

- Factor analysis of CEC data performed using R package psych
- Accelerated aging estimates obtained via R package DuandinPACE
- Methylation Risk Scores (MRSs) derived using machine-learning.



* Hoffman et al. (2011)

Demographics of Study Participants

	Breachers	Grenade Operators
Number of subjects	190	30
Age in years, mean (SD)	30.43 (5.65)	33.21 (5.37)
Duration of Service in years, mean (SD)	9.42 (4.80)	13.45 (5.27)
History of mTBI, No. (%)		
Yes	128 (67.4%)	22 (73.3%)
No	62 (32.6%)	8 (26.7%)

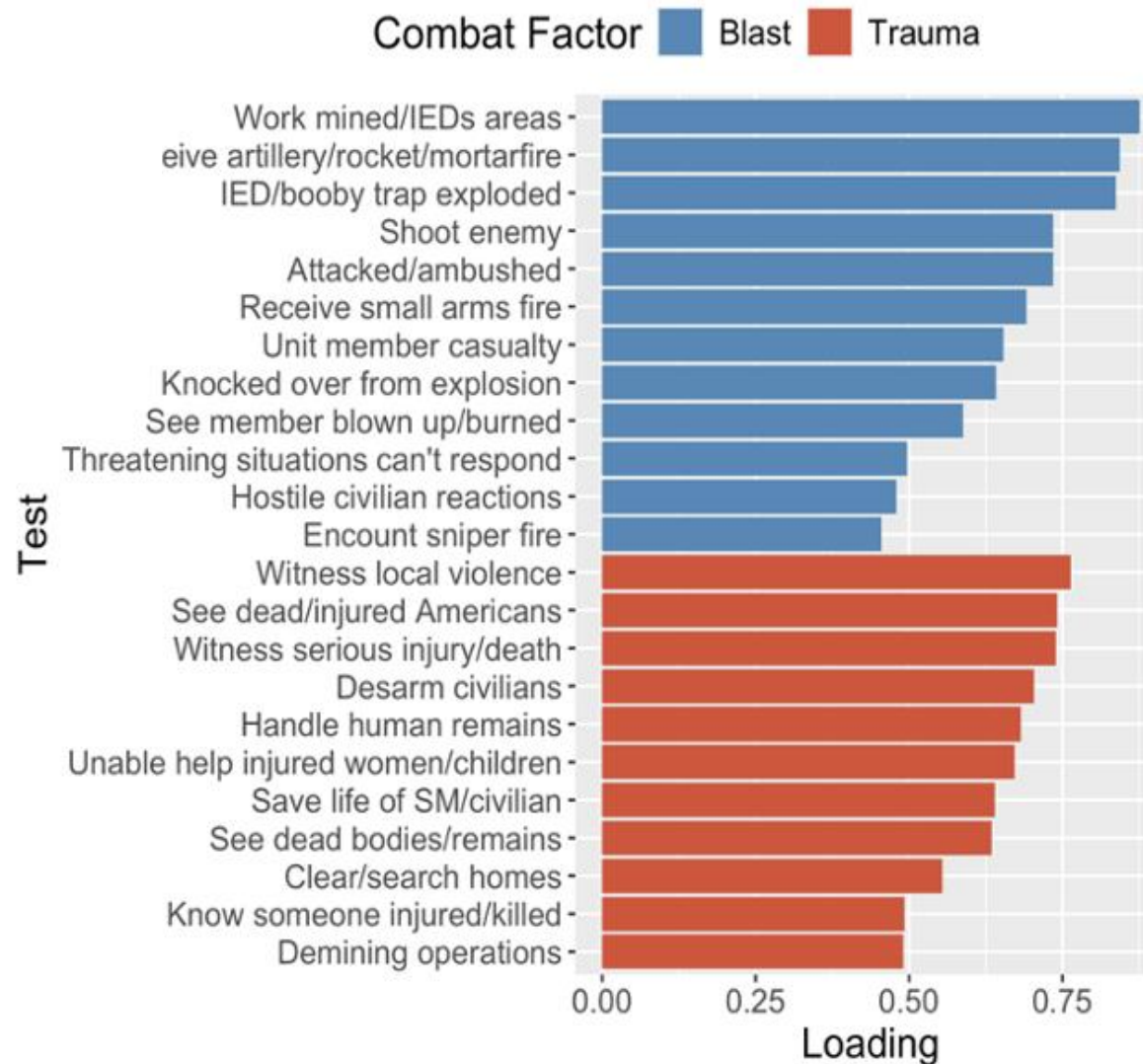
Representative Questions of the 33-item Combat Exposure Checklist (CES)

	Never	One Time	Two to Four Times	Five to Nine Times	Ten or More Times
Being attacked or ambushed					
Seeing destroyed homes and villages					
Receiving small arms fire					
Witnessing an accident which resulted in serious injury or death					
Witnessing violence within the local population or between ethnic groups					
Seeing dead or seriously injured Americans					
Knowing someone seriously injured or killed					
Participating in demining operations					
Improvised explosive device (IED)/booby trap exploded near you					
Working in areas that were mined or had IED's					
Having hostile reactions from civilians					
Disarming civilians					

Additional question was added to capture participants' prior blast experiences (item #34):

“Have you ever been physically moved or knocked over from an explosion?”

Results of Factor Analysis for Combat Exposure Scale



Correlation of Combat Factors with Reported Medical History

0.31	0.23	Career total blast
0.32	0.31	Asleep
0.3	0.24	RingEars
0.27	0.26	Concussion
0.23	0.2	Memory
0.21	0.18	Backache
0.18	0.22	Psychological
0.18	0.2	LightHeadedness
		Irritability
	0.22	Avoid stress
	0.23	Reaction stress
CF1-Blast	CF2-Trauma	

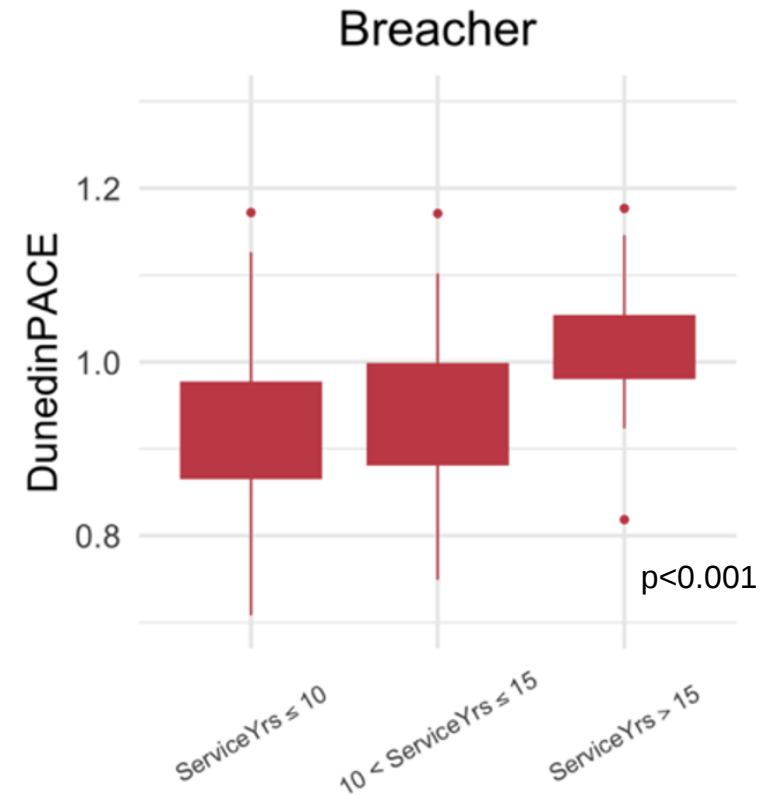
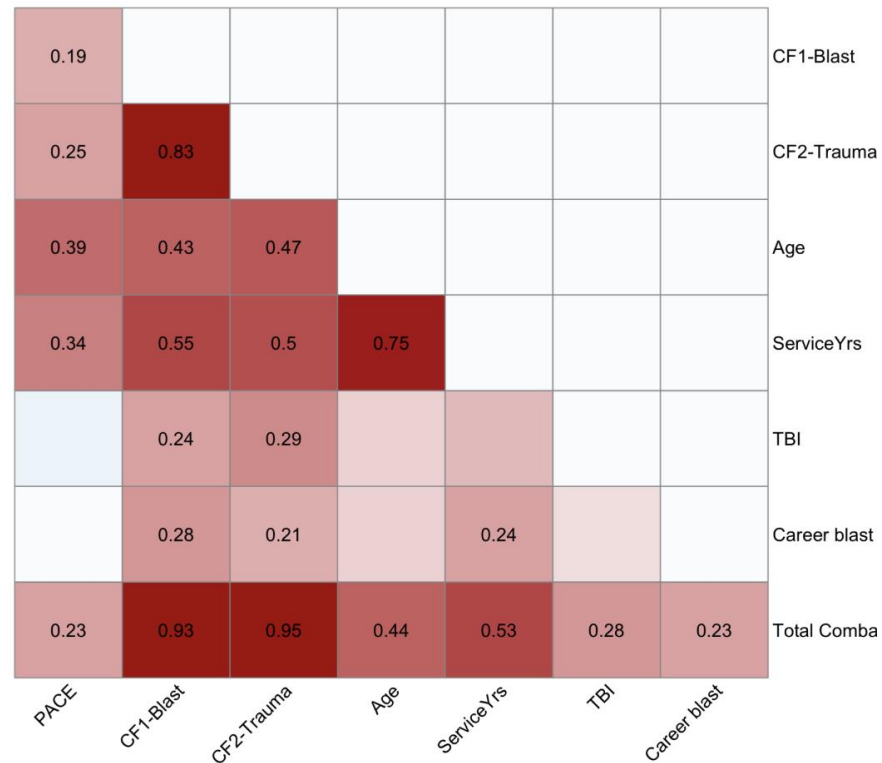
- “Ringing in ears” more strongly correlated with physical-blast exposure factor
- “Avoiding activities or situations that remind you of a stressful experience” and “Physical reaction (heart pounding, sweating)” when reminded of a stressful experience more strongly correlated with psychological-trauma exposure factor

Epigenetic Age Acceleration (EAA)

- Higher epigenetic age acceleration is associated with increased risk of all-cause mortality, functional and cognitive decline, as well as chronic disease burden.
- Epigenetic clocks measure age-related methylation changes using methylation levels at CpG dinucleotides across the genome.
- DunedinPACE (an epigenetic clock) was developed by training on clinical phenotypes, mortality, longitudinal functional decline, and thus can measure health-related biological aging and pace of aging.

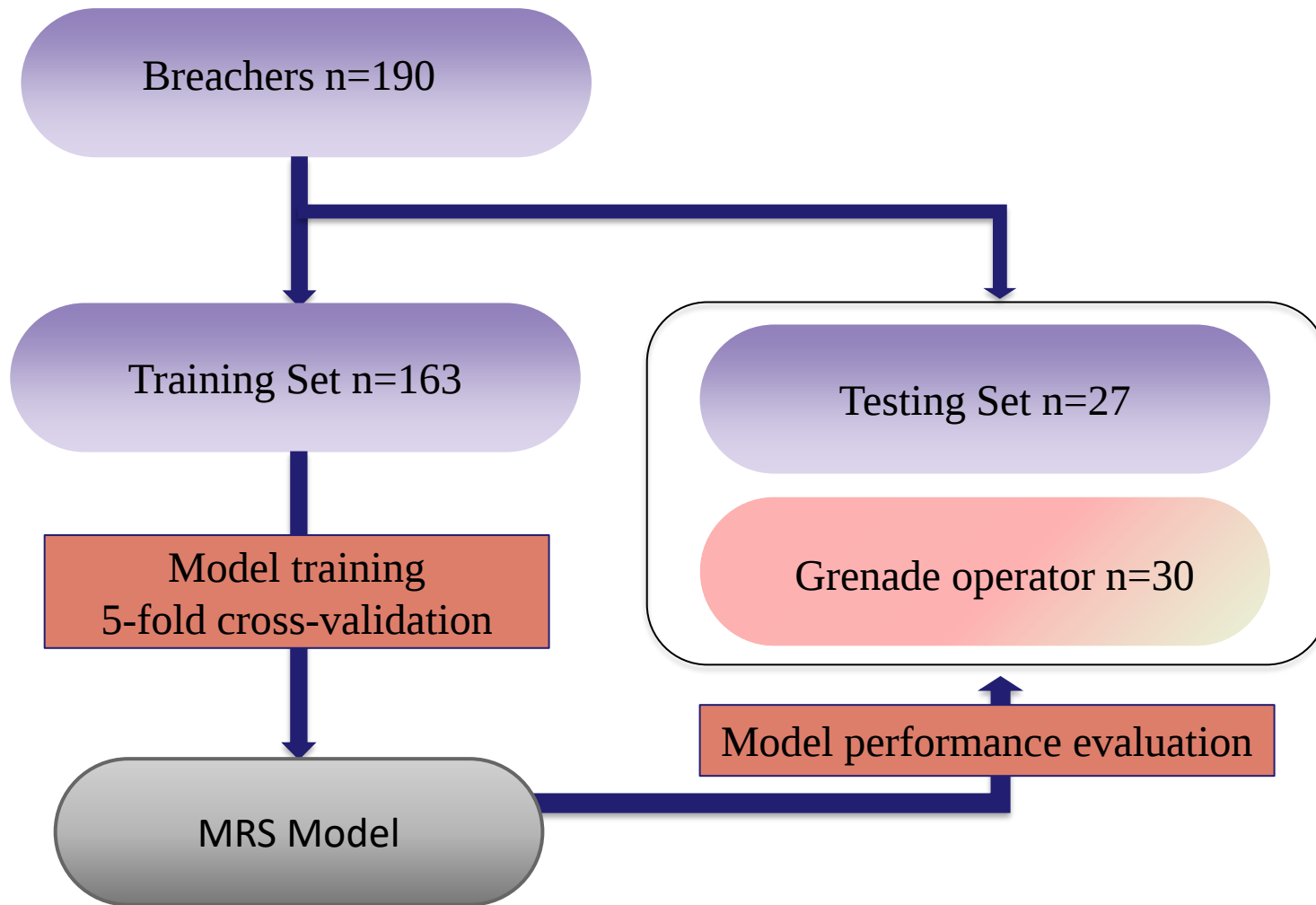
Question: Does EAA associate with military service exposures?

Accelerated epigenetic aging associated with combat related blast and psychological trauma exposures



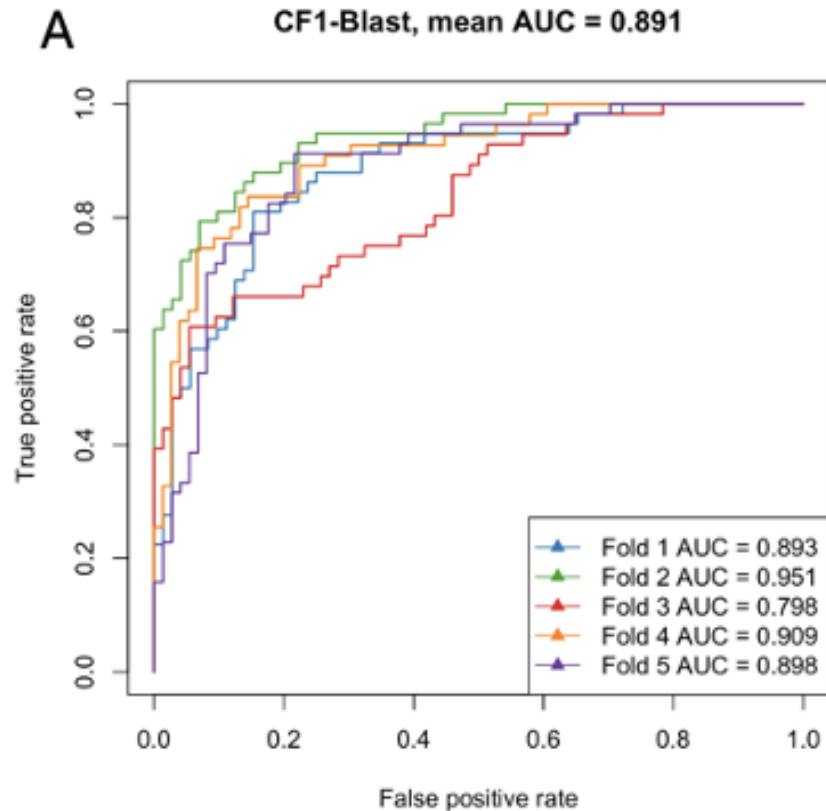
- TBI positively associates with physical/blast and psychological trauma combat exposures
- Physical-blast and psychological trauma combat exposures positively associate with epigenetic age acceleration.
- Increasing years in military services significantly correlates with higher rate of epigenetic age acceleration.

Development of MRS models using machine learning



- LASSO (Least Absolute Shrinkage and Selection Operator) used to develop MRS models predicting combat factors using DNA methylation data.
- Data was split into data sets:
 - Training (breacher: 86%, n=163)
 - Testing (breacher: 14%, n=27; grenade operators: 100%, n=30).
- Performed five-fold cross-validation within training dataset to optimize parameters for best prediction accuracy.
- Model performance evaluated using area under the curve (AUC).

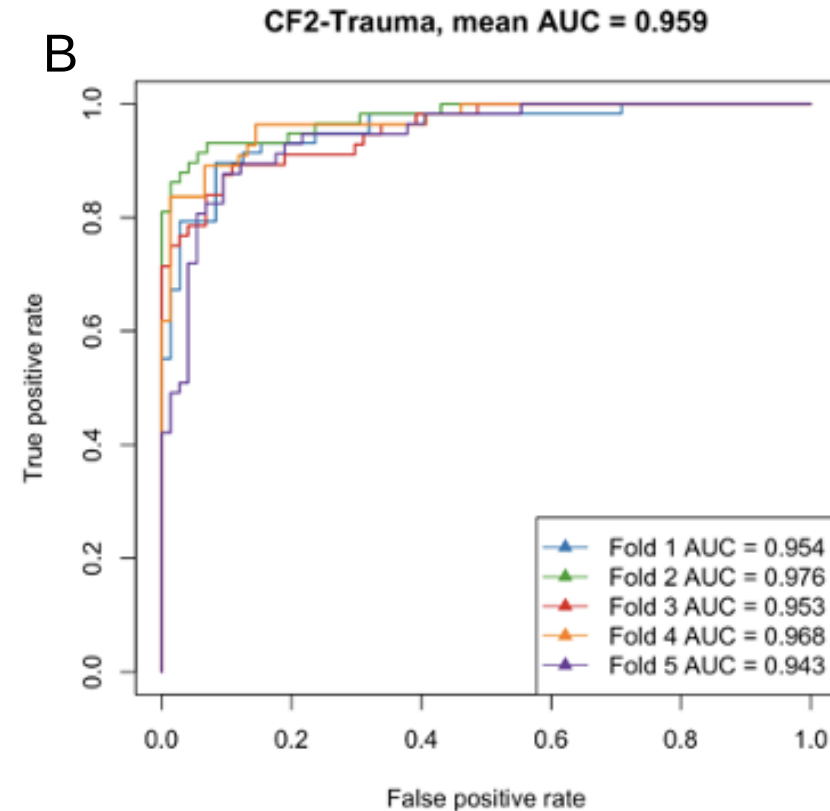
Blast and trauma methylation risk scores, MRS, Training and validation



External Validation: AUC

Breacher = 74%

Grenade = 52%

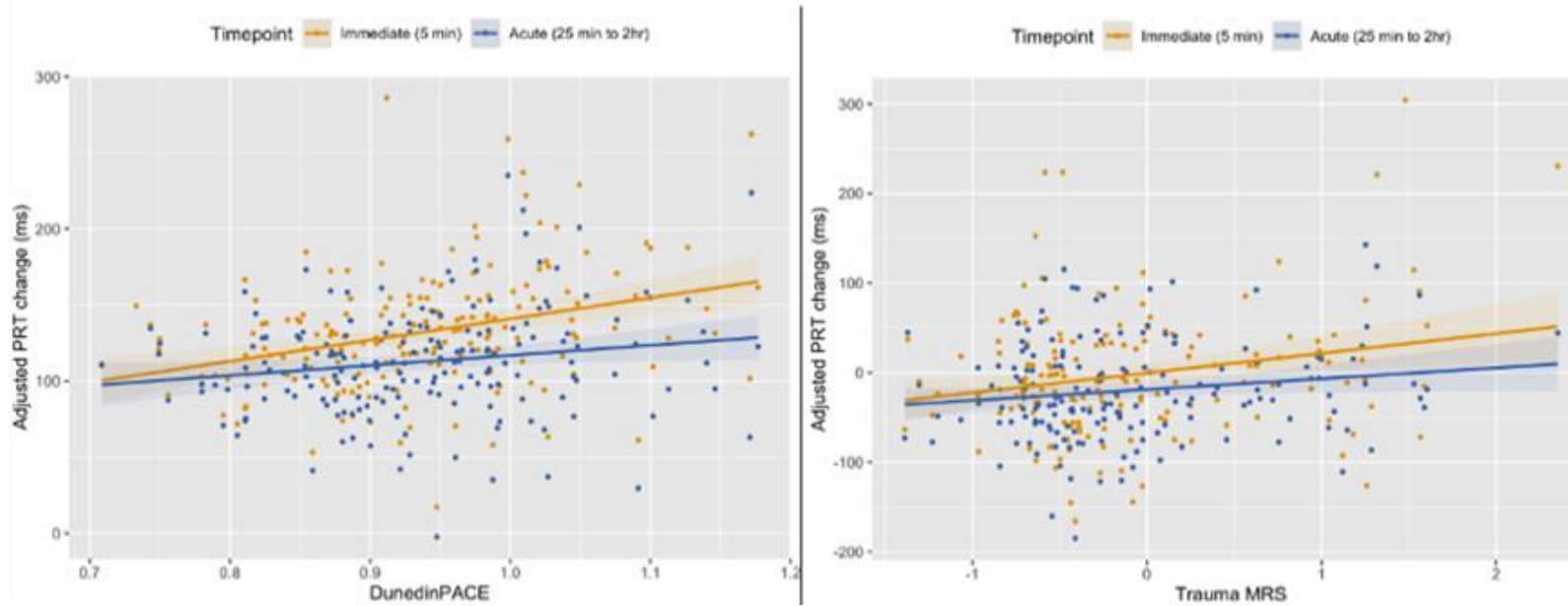


External Validation: AUC

Breacher = 74%

Grenade = 72%

Neurocognitive performance for breachers during military tactical training



- Epigenetic accelerated aging (EAA) measured by DunedinPace vs. change in procedural reaction time (PRT) following blast exposure during tactical training, at immediate (within 5 minutes) and acute (25 minutes to 2 hours) timepoints.
- Data show accelerated aging and prior history of psychological trauma during combat is significantly associated with diminished neurocognitive performance during tactical operations immediately but not acutely post-blast.
- CF2-Trauma methylation risk score vs. change in procedural reaction time (PRT) following blast exposure during tactical training, at immediate (within 5 minutes) and acute (25 minutes to 2 hours) timepoints.

Conclusions and future directions

- Findings in Warfighters evidence accelerated epigenetic aging related to duration of military service, as well as blast and trauma exposures.
- Findings in Warfighters identify altered DNA methylation patterns associated with blast and psychological trauma exposures proximal to regulatory regions of genes previously implicated in neuroinflammation, blood-brain barrier integrity, and auditory functioning.
- Application of machine learning methods to predict blast and psychological trauma exposure in Warfighters using Methylation Risk Scores showed acceptable prediction accuracy for psychological trauma exposures.
- MRS encapsulating psychological trauma exposure significantly associated with diminished Neurocognitive performance in Warfighters following blast exposure while engaged in tactical trainings.
- Epigenetic age acceleration also significantly associated with diminished neurocognitive performance in Warfighters following blast exposure during training operations.

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